Only four genes (EDA1, EDAR, EDARADD and WNT10A) account for 90 % of hypohidrotic/anhidrotic ectodermal dysplasia cases

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Human Mutation

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Journal:	*Human Mutation*
Manuscript ID:	humu-2010-0289.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	17-Sep-2010
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Key Words:	HED/EDA, EDA1, EDAR, EDARADD, WNT10A

Research Article

Only four genes (*EDA1, EDAR, EDARADD* and *WNT10A*) account for 90 % of hypohidrotic/anhidrotic ectodermal dysplasia cases

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Abstract

Hypohidrotic and anhidrotic ectodermal dysplasia (HED/EDA) is a rare genodermatosis characterized by abnormal development of sweat glands, teeth and hair. Three disease causing genes have been hitherto identified, namely *i*) *EDA1* accounting for X-linked forms, *ii*) *EDAR*, and *iii*) *EDARADD*, causing both autosomal dominant and recessive forms. Recently, *WNT10A* gene was identified as responsible for various autosomal recessive forms of ectodermal dysplasias, including onycho-odonto-dermal dysplasia (OODD) and Schöpf-Schulz-Passarge syndrome. We systematically studied *EDA1*, *EDAR*, *EDARADD* and *WNT10A* genes in a large cohort of 65 unrelated patients, of which 61 presented with HED/EDA. A total of 50 mutations (including 32 novel mutations) accounted for 60/65 cases in our series. These four genes accounted for 92 % (56/61 patients) of HED/EDA cases: *i*) *EDA1* gene was the most common disease causing gene (58 % of cases), *ii*) *WNT10A* and *EDAR* were each responsible for 16 % of cases. Moreover, a novel disease locus for dominant HED/EDA mapped to chromosome 14q12-q13.1. While no clinical differences between patients carrying *EDA1*, *EDAR* or *EDARADD* mutations could be identified, patients harboring *WNT10A* mutations displayed distinctive clinical features (marked dental phenotype, no facial dysmorphism), helping to decide which gene should be first investigated in HED/EDA.

Keywords: HED/EDA; *EDA1*; *EDAR*; *EDARADD*; *WNT10A*.

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Introduction

Ectodermal dysplasia (ED) is a clinically and genetically heterogeneous condition characterized by abnormal development of two or more of the following ectodermal-derived structures: hair, teeth, nails, and sweat glands [Freire-Maia, 1977; Lamartine, 2003; Visinoni, et al., 2009]. Anomalies in other organs and systems may also be observed [Pinheiro and Freire-Maia, 1994; Dhanrajani and Jiffry, 1998; Priolo, et al., 2000]. Anhidrotic or hypohidrotic ectodermal dysplasia (HED/EDA), the most common phenotype of ED, is characterized by a triad of signs comprising sparse hair (hypotrichosis), abnormal or missing teeth (anodontia or hypodontia) and inability to sweat (anhidrosis or hypohidrosis). Typical clinical manifestations also include dryness of the skin, eyes, airways and mucous membranes presumably due to the defective development of several exocrine glands. HED/EDA can be associated with dysmorphic features (forehead bumps, rings under the eyes, everted nose and prominent lips) and occasionally with absent nipples.

The most frequent form of HED/EDA (MIM305100) results from mutations in the *EDA1* gene, located on chromosome Xq12-q13.1 and encoding ectodysplasin (MIM300451), a member of the Tumor Necrosis Factor (TNF) family [Kere, et al., 1996; Bayes, et al., 1998]. Mutations in the EDA receptor encoding gene *EDAR*, located on chromosome 2q11-q13 (MIM604095), or in the EDAR-Associated Death Domain encoding gene *EDARADD*, located on chromosome 1q42-q43 (MIM606603), have been shown to cause autosomal recessive and dominant HED forms respectively [Monreal, et al., 1999; Headon, et al., 2001; Bal, et al., 2007]. These three forms are clinically indistinguishable, probably because they alter a single signal transduction pathway. Indeed, the binding of ectodysplasin to its receptor, EDAR, allows the recruitment of EDARADD as an adapter to activate the NF-κB signalling pathway [Yan, et al., 2000; Koppinen, et al., 2001; Kumar, et al., 2001]. This pathway is necessary for initiation, formation and differentiation of

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skin appendages [Mikkola, et al., 1999; Laurikkala, et al., 2002; Mustonen, et al., 2004; Mou, et al., 2006; Schmidt-Ullrich, et al., 2006; Pummila, et al., 2007].

On the other hand, loss-of-function and missense mutations in the *WNT10A* gene (chromosome 2q35, MIM257980) have been shown to cause odonto-onycho-dermal dysplasia, a rare form of ectodermal dysplasia [Adaimy, et al., 2007; Nawaz, et al., 2009]. Subsequently, *WNT10A* mutations have been reported in various forms of ectodermal dysplasia, including in three patients with sweating anomalies [Bohring, et al., 2009; Nagy, et al., 2010; van Geel, et al., 2010].

In order to evaluate the impact of *EDA1*, *EDAR*, *EDARADD* and *WNT10A* mutations in HED/EDA, we have sequenced all four genes in a large cohort of 65 unrelated patients. Among them, 61 patients had HED/EDA. Our study shows that only four genes account for more than 90 % of cases in HED/EDA.

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Patients and Methods

Patients

Since 2002, a total of 65 patients were recruited by the departments of Dermatology and Genetics of Necker-Enfants Malades Hospital. Patients included in this study presented abnormalities of at least two of the three following ectodermal structures: teeth, hair and sweat glands. Most of those patients (53/65) presented the classical triad of HED/EDA phenotype. The relatives of the probands occasionally presented abnormalities of only one epidermal structure. The great majority of cases were familial cases (48/65) of Caucasian origin (45/65). A total of 9/65 patients were born to related parents. Informed consent for DNA analysis and reproduction of the photographs was obtained from all individuals concerned.

Mutation detection

DNA was extracted from patients' blood leukocytes using the Illustra DNA extraction kit BACC3 (GE Healthcare), following manufacturer's instructions. All exons and at least 60 base pair of flanking intronic sequences of the *EDA1* (NM_001399.4), *EDAR* (NM_022336.3), *EDARADD* (NM_080738.3) and *WNT10A* genes (NM_025216.2) were amplified by PCR using specific primers (see Supp. Table S1). Both DNA strands were sequenced using the Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems). Sequence variations were numbered with +1 corresponding to the A of the ATG translation initiation codon in the cDNA reference sequence, or with the initiation codon as codon 1 in the proteic reference sequence, according to journal guidelines (www.hgvs.org/mutnomen).

For Quantitative Multiplex PCR of Short fluorescent Fragments analysis (QMPSF), exon 18 of *MLH1* gene (E. coli MutL Homolog 1) or exon 3 of *GFAP* gene (Glial Fibrillary Acidic Protein) were amplified as positive controls, along with each exon of the *WNT10A* gene (see Supp. Table S1). PCR products were migrated using an ABI3130 sequencer (Applied Biosystems), and analyzed with GeneScan Analysis software version 3.7 (Applied Biosystems).

A genome-wide scan for one large family carrying no known mutation was undertaken with 382 pairs of fluorescent oligonucleotides of the Genscan Linkage Mapping set, version II (average spacing of 10 cM, Perkin-Elmer Cetus) under conditions recommended by the manufacturer. To refine the novel HED locus identified, additional microsatellites DNA markers from chromosome 14q region were studied. After amplification, PCR products were pooled with GeneScan 400D ROX size standard ladder (0.3 µl; Applied Biosystems), and analyzed on an ABI3130 sequencer.

Results

A total of 31 *EDA1* mutations, including 20 novel mutations were identified in 35/65 cases of our cohort (Table 1, Figure 1A, Supp. Figure S1A). Most of those mutations were missense mutations (24/31), located in exons 3 and 5 to 9. The male patients carrying *EDA1* mutations

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were severely affected, as they all displayed anomalies in the three ectodermal structures. Carrier females were occasionally moderately affected, but two displayed unusually severe symptoms (patients EDA1-F19 and EDA1-F24, Figure 1B1-2).

As to the *EDAR* gene, a total of 10 mutations, including 5 novel mutations, were identified in 10/65 patients (Table 1, Figure 1A, Supp. Figure S1B). Five mutations were dominantly, and five recessively inherited. They were mostly located in exons 9 to 12 (7/10).

Interestingly, the dominant mutations were all located close to the EDAR Death Domain (DD, two missense mutations, one single residue duplication and two frameshift mutations).

Clinical features of these patients were indistinguishable from those observed in *EDA1* patients. However, individuals carrying dominant *EDAR* mutations were less severely affected than their recessive counterparts (Figure 1C and D). This was particularly true with respect to sweating (Table 1).

As to the *EDARADD* gene, only one novel dominantly inherited missense mutation was found in a patient with a moderate HED phenotype (NM_080738.3: c.328G>T, p.D110Y; Table 1, Figure 1E1-2). This mutation located near the EDARADD Death Domain (Supp. Figure S1C), probably altered its interaction with EDAR and/or multimerization of EDARADD.

As to the *WNT10A* gene, a total of 8 mutations were identified in 14/65 cases (9 familial and 5 sporadic cases; Table 2, Figure 2, and Supp. Figure S1D). They included 5 novel missense mutations and the first *WNT10A* duplication identified to date. The p.F228I mutation was prevalent in our cohort (10/14 patients) and found in homozygote or compound heterozygote patients. None of those mutations were found in 150 control chromosomes from healthy Caucasian individuals. QMPSF analysis of the four exons of the *WNT10A* gene in six heterozygote patients failed to detect any intragenic deletion (not shown).

Interestingly, most patients carrying *WNT10A* mutations (10/14) presented with sweating anomalies, including total anhidrosis for two of them (WNT10A-F03 and S01). However,

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comparison of those patients with HED/EDA cases harboring mutations in the ectodysplasin pathway allowed us to identify slight differences in their respective phenotypes. Indeed, dermatological features (anomalies of hair and sweat glands) were less severe and none of the patients carrying *WNT10A* mutations presented facial dysmorphism. Their dental phenotype consisted in microdontia, while teeth agenesis was more frequent in patients carrying mutations in the ectodysplasin pathway (Table 2, Figure 1F1-3 and 1G). The other four patients with *WNT10A* mutations presented with incomplete form of OODD or unclassified form of ED (Table 2).

Finally, among the 5/65 unexplained cases of our cohort, one patient was the proband of a four-generation French family, presenting an autosomal dominant HED (patient HED-F01, Supp. Table S2). Genome-wide scan analysis led to the mapping of this novel disease gene to a 5 cM interval on chromosome 14q12-q13.1 (Zmax = 4.8; Supp. Figure S3). The strongest candidate genes have been excluded by sequencing analysis (*PAX9*, *NFKBIA*, *PSMA6*, *SNX6*, *MBIP*). None of the remaining known genes mapping to this region were involved in either ectodysplasin or Wnt signalling pathways.

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Discussion

Here we show that four genes (*EDA1*, *EDAR*, *EDARADD* and *WNT10A*) accounted for 92 % of cases in a series of 65 unrelated patients, of which 61 presented with HED/EDA. *EDA1* mutations were prevalent (58 % of cases). *WNT10A* mutations were more frequent than *EDAR* mutations in our cohort, but both genes are each responsible for 16 % of HED/EDA cases (Figure 1A). We also report here on a novel disease gene mapping to chromosome 14q12-q13.1 and accounting for autosomal dominant HED/EDA.

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The phenotypes associated with *EDA1*, *EDAR*, and *EDARADD* mutations were indistinguishable and consistently included hypohidrosis or anhidrosis, sparse hair, and oligodontia with abnormal

conical teeth, frequently associated with dryness of skin, eczema and facial dysmorphism. Yet, the clinical expression of *WNT10A* mutations was highly variable. *WNT10A* gene was first involved in OODD syndrome (MIM257980) and subsequently in Schöpf-Schulz-Passarge syndrome (MIM224750) [Adaimy, et al., 2007; Bohring, et al., 2009]. We confirmed the involvement of *WNT10A* gene in OODD or OODD-like forms of ED (3/14 patients), and unclassified ED (1/14; Table 2). Moreover, 10/14 patients of our cohort harboring *WNT10A* mutations presented with anomalies corresponding to HED/EDA, *ie* hypohidrosis or anhidrosis associated with teeth and hair anomalies. Thus our results expanded the spectrum of *WNT10A* mutations to HED/EDA phenotype. The variability of phenotypes associated to *WNT10A* mutations is not understood yet. The type of mutations (nonsense or missense) and their functional effects could explain this broad spectrum of phenotypes.

Our study gives support to the high incidence of *EDA1* mutations (35/61, 58 %), and the scarcity of *EDAR* mutations in HED/EDA (10/61, 16 %) [Vincent, et al., 2001; Chassaing, et al., 2006; van der Hout, et al., 2008]. Interestingly, while *WNT10A* was reportedly known to account for various forms of ED, we provide here the first evidence for its very high incidence in HED/EDA cases (10/61, 16 %) [Adaimy, et al., 2007; Bohring, et al., 2009; Nawaz, et al., 2009; Nagy, et al., 2010].

Yet, the mode of inheritance of *WNT10A* mutations remains unclear. While eight patients were homozygotes or compound heterozygotes, only one heterozygous mutation was identified in six patients. Although a single mutation could probably explain the moderate cases (WNT10A-F08, –F09 and –S04), the three severely affected patients may carry a second unidentified mutation (in intronic or regulatory sequences of *WNT10A*). Alternatively, a dominant mode of inheritance with variable penetrance could also explain the severe phenotype of these patients.

A higher proportion of tooth anomalies has been previously described in males harboring heterozygous *WNT10A* mutations [Bohring, et al., 2009]. These sex-biased manifestations were

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not found in our cohort. Dental abnormalities were the most frequent manifestation in heterozygous patients in both sexes. This difference may result from the high frequency of the p.C107X mutation in the German/Turkish cohort [Bohring, et al., 2009].

Most of the HED mutations reported here were missense mutations, located in functionally important domains of the protein (furin clivage site, collageneous and TNF domains for Ectodysplasin; Death Domain for EDAR and EDARADD). Interestingly, all dominant mutations reported to date in EDAR were located in its C-terminal region near its Death Domain. These mutations probably exert a dominant negative effect on the wild-type allele *via* the formation of non-functional multimers, unable to recruit EDARADD. All missense EDARADD mutations are located in (or very close to) the Death Domain of EDARADD, probably impairing its interaction with EDAR.

The involvement of the ectodysplasin/NF-κB and Wnt/β-catenin pathways in ectodermal appendage development has long been known [Kere, et al., 1996; Gat, et al., 1998]. Because the two pathways are involved in early steps of ectodermal placode development, one can hypothesize that the 10 % hitherto unknown mutations may lie in either of these two pathways.

In conclusion, *WNT10A* gene should be considered as a candidate gene for HED/EDA, especially in the clinical condition of microdontia, sweating anomalies, and absence of facial dysmorphism. Alternatively, patients with the classical triad of HED/EDA signs and facial dysmorphism, should be first studied for *EDA1* gene, except in non-X-linked familial cases. If no mutation is identified in *EDA1*, *EDAR* is the next gene to consider, before *WNT10A* and *EDARADD*.

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Acknowledgments

Authors declare no conflict of interest. The authors would like to thank all patients and families for their participation in this study. This work was supported by Agence Nationale de la

Recherche (ANR) and Programme Hospitalier de Recherches Cliniques (PHRC) grants, and by Fondation pour la Recherche Médicale (PhD student grant to C.C).

References

Adaimy, L, Chouery, E, Megarbane, H, Mroueh, S, Delague, V, Nicolas, E, Belguith, H, de Mazancourt, P and Megarbane, A. 2007. Mutation in WNT10A is associated with an autosomal recessive ectodermal dysplasia: the odonto-onycho-dermal dysplasia. Am J Hum Genet 81:821-8.

Bal, E, Baala, L, Cluzeau, C, El Kerch, F, Ouldim, K, Hadj-Rabia, S, Bodemer, C, Munnich, A, Courtois, G, Sefiani, A and Smahi, A. 2007. Autosomal dominant anhidrotic ectodermal dysplasias at the EDARADD locus. Hum Mutat 28:703-9.

Bayes, M, Hartung, AJ, Ezer, S, Pispa, J, Thesleff, I, Srivastava, AK and Kere, J. 1998. The anhidrotic ectodermal dysplasia gene (EDA) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. Hum Mol Genet 7:1661-9.

Bohring, A, Stamm, T, Spaich, C, Haase, C, Spree, K, Hehr, U, Hoffmann, M, Ledig, S, Sel, S, Wieacker, P and Ropke, A. 2009. WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. Am J Hum Genet 85:97-105.

Chassaing, N, Bourthoumieu, S, Cossee, M, Calvas, P and Vincent, MC. 2006. Mutations in EDAR account for one-quarter of non-ED1-related hypohidrotic ectodermal dysplasia. Hum Mutat 27:255-9.

Dhanrajani, PJ and Jiffry, AO. 1998. Management of ectodermal dysplasia: a literature review. Dent Update 25:73-5.

Freire-Maia, N. 1977. Ectodermal dysplasias revisited. Acta Genet Med Gemellol (Roma) 26:121-31.

Gat, U, DasGupta, R, Degenstein, L and Fuchs, E. 1998. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 95:605-14.

Headon, DJ, Emmal, SA, Ferguson, BM, Tucker, AS, Justice, MJ, Sharpe, PT, Zonana, J and Overbeek, PA. 2001. Gene defect in ectodermal dysplasia implicates a death domain adapter in development. Nature 414:913-6.

Kere, J, Srivastava, AK, Montonen, O, Zonana, J, Thomas, N, Ferguson, B, Munoz, F, Morgan, D, Clarke, A, Baybayan, P, Chen, EY, Ezer, S, Saarialho-Kere, U, de la Chapelle, A and Schlessinger, D. 1996. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. Nat Genet 13:409-16.

Koppinen, P, Pispa, J, Laurikkala, J, Thesleff, I and Mikkola, ML. 2001. Signaling and subcellular localization of the TNF receptor Edar. Exp Cell Res 269:180-92.

Kumar, A, Eby, MT, Sinha, S, Jasmin, A and Chaudhary, PM. 2001. The ectodermal dysplasia receptor activates the nuclear factor-kappaB, JNK, and cell death pathways and binds to ectodysplasin A. J Biol Chem 276:2668-77.

Lamartine, J. 2003. Towards a new classification of ectodermal dysplasias. Clin Exp Dermatol 28:351-5.

Laurikkala, J, Pispa, J, Jung, HS, Nieminen, P, Mikkola, M, Wang, X, Saarialho-Kere, U, Galceran, J, Grosschedl, R and Thesleff, I. 2002. Regulation of hair follicle development by the TNF signal ectodysplasin and its receptor Edar. Development 129:2541-53.

Mikkola, ML, Pispa, J, Pekkanen, M, Paulin, L, Nieminen, P, Kere, J and Thesleff, I. 1999. Ectodysplasin, a protein required for epithelial morphogenesis, is a novel TNF homologue and promotes cell-matrix adhesion. Mech Dev 88:133-46.

Monreal, AW, Ferguson, BM, Headon, DJ, Street, SL, Overbeek, PA and Zonana, J. 1999. Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. Nat Genet 22:366-9.

Mou, C, Jackson, B, Schneider, P, Overbeek, PA and Headon, DJ. 2006. Generation of the primary hair follicle pattern. Proc Natl Acad Sci U S A 103:9075-80.

Mustonen, T, Ilmonen, M, Pummila, M, Kangas, AT, Laurikkala, J, Jaatinen, R, Pispa, J, Gaide, O, Schneider, P, Thesleff, I and Mikkola, ML. 2004. Ectodysplasin A1 promotes placodal cell fate during early morphogenesis of ectodermal appendages. Development 131:4907-19.

Nagy, N, Wedgeworth, E, Hamada, T, White, JM, Hashimoto, T and McGrath, JA. 2010. Schopf-Schulz-Passarge syndrome resulting from a homozygous nonsense mutation in WNT10A. J Dermatol Sci 58:220-2.

Nawaz, S, Klar, J, Wajid, M, Aslam, M, Tariq, M, Schuster, J, Baig, SM and Dahl, N. 2009. WNT10A missense mutation associated with a complete odonto-onycho-dermal dysplasia syndrome. Eur J Hum Genet 17:1600-5.

Pinheiro, M and Freire-Maia, N. 1994. Ectodermal dysplasias: a clinical classification and a causal review. Am J Med Genet 53:153-62.

Priolo, M, Silengo, M, Lerone, M and Ravazzolo, R. 2000. Ectodermal dysplasias: not only 'skin' deep. Clin Genet 58:415-30.

Pummila, M, Fliniaux, I, Jaatinen, R, James, MJ, Laurikkala, J, Schneider, P, Thesleff, I and Mikkola, ML. 2007. Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of Bmp activity and induction of Shh expression. Development 134:117-25.

Schmidt-Ullrich, R, Tobin, DJ, Lenhard, D, Schneider, P, Paus, R and Scheidereit, C. 2006. NF-kappaB transmits Eda A1/EdaR signalling to activate Shh and cyclin D1 expression, and controls post-initiation hair placode down growth. Development 133:1045-57.

van der Hout, AH, Oudesluijs, GG, Venema, A, Verheij, JB, Mol, BG, Rump, P, Brunner, HG, Vos, YJ and van Essen, AJ. 2008. Mutation screening of the Ectodysplasin-A receptor gene EDAR in hypohidrotic ectodermal dysplasia. Eur J Hum Genet 16:673-9.

van Geel, M, Gattas, M, Kesler, Y, Tong, P, Yan, H, Tran, K, Steijlen, PM, Murrell, DF and van Steensel, MA. 2010. Phenotypic variability associated with WNT10A nonsense mutations. Br J Dermatol

Vincent, MC, Biancalana, V, Ginisty, D, Mandel, JL and Calvas, P. 2001. Mutational spectrum of the ED1 gene in X-linked hypohidrotic ectodermal dysplasia. Eur J Hum Genet 9:355-63.

Visinoni, AF, Lisboa-Costa, T, Pagnan, NA and Chautard-Freire-Maia, EA. 2009. Ectodermal dysplasias: clinical and molecular review. Am J Med Genet A 149A:1980-2002.

Yan, M, Wang, LC, Hymowitz, SG, Schilbach, S, Lee, J, Goddard, A, de Vos, AM, Gao, WQ and Dixit, VM. 2000. Two-amino acid molecular switch in an epithelial morphogen that regulates binding to two distinct receptors. Science 290:523-7.

Deleted: Adaimy L, Chouery E, Megarbane H, Mroueh S, Delague V, Nicolas E, Belguith H, de Mazancourt P, Megarbane A. 2007. Mutation in WNT10A is associated with an autosomal recessive ectodermal dysplasia: the odonto-onycho-dermal dysplasia. Am J Hum Genet 81(4):821-8.¶

Bal E, Baala L, Cluzeau C, El Kerch F, Ouldim K, Hadj-Rabia S, Bodemer C, Munnich A, Courtois G, Sefiani A and others. 2007. Autosomal dominant anhidrotic ectodermal dysplasias at the EDARADD locus. Hum Mutat 28(7):703-9.¶

Bayes M, Hartung AJ, Ezer S, Pispa J, Thesleff I, Srivastava AK, Kere J. 1998. The anhidrotic ectodermal dysplasia gene (EDA) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. Hum Mol Genet 7(11):1661-9.¶

Bohring A, Stamm T, Spaich C, Haase C, Spree K, Hehr U, Hoffmann M, Ledig S, Sel S, Wieacker P and others. 2009. WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. Am J Hum Genet 85(1):97-105.¶

Chassaing N, Bourthoumieu S, Cossee M, Calvas P, Vincent MC. 2006. Mutations in EDAR account for one-quarter of non-ED1-related hypohidrotic ectodermal dysplasia. Hum Mutat 27(3):255-9.¶

Dhanrajani PJ, Jiffry AO. 1998. Management of ectodermal dysplasia: a literature review. Dent Update 25(2):73-5.¶

Freire-Maia N. 1977. Ectodermal dysplasias revisited. Acta Genet Med Gemellol (Roma) 26(2):121-31.¶

Gat U, DasGupta R, Degenstein L, Fuchs E. 1998. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 95(5):605-14.¶

Gunadi, Miura K, Ohta M, Sugano A, Lee MJ, Sato Y, Matsunaga A, Hayashi K, Horikawa T, Miki K and others. 2009. Two novel mutations in the ED1 gene in Japanese families with X-linked hypohidrotic ectodermal dysplasia. Pediatr Res 65(4):453-7.¶

Headon DJ, Emmal SA, Ferguson BM, Tucker AS, Justice MJ, Sharpe PT, Zonana J, Overbeek PA. 2001. Gene defect in ectodermal dysplasia implicates a death domain adapter in development. Nature 414(6866):913-6.¶

Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B, Munoz F, Morgan D, Clarke A, Baybayan P and others. 1996. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. Nat Genet 13(4):409-16.¶

Koppinen P, Pispa J, Laurikkala J, Thesleff I, Mikkola ML. 2001. Signaling and subcellular localization of the TNF receptor Edar. Exp Cell Res 269(2):180-92.¶

Kumar A, Eby MT, Sinha S, Jasmin A, Chaudhary PM. 2001. The ectoder ... [1]

Legends to figures

Figure 1: Mutation distribution and clinical appearance of HED/EDA patients.

A: Frequencies of identified mutations for each known gene in our series of 61 HED/EDA patients. B1-B4: severely affected female patient (EDA1-F19) at age 6 years (B1, B2) and her brother at age 5 years (B3, B4). C: severely affected patient EDAR-F01 at age 3 years. D: moderately affected patient EDAR-F05 at age 30 years. E1-E2: EDARADD-F01 at age 9 years. F1-F3: WNT10A-F03 at age 16 years. G: WNT10A-S04 at age 5 years. Pictures collection from the department of Dermatology, Necker-Enfants Malades Hospital, Paris.

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Figure 2: Pedigrees of the 9 families carrying mutations in *WNT10A* gene.

Mutations identified in each individual are indicated, WT designated a second wild-type allele. Filled symbols represented severely affected individuals, half-filled symbols moderately affected individuals. The question mark (?) indicated an undetermined status, an asterisk (*): no DNA available.

Adaimy L, Chouery E, Megarbane H, Mroueh S, Delague V, Nicolas E, Belguith H, de Mazancourt P, Megarbane A. 2007. Mutation in WNT10A is associated with an autosomal recessive ectodermal dysplasia: the odonto-onycho-dermal dysplasia. Am J Hum Genet 81(4):821-8.

Bal E, Baala L, Cluzeau C, El Kerch F, Ouldim K, Hadj-Rabia S, Bodemer C, Munnich A, Courtois G, Sefiani A and others. 2007. Autosomal dominant anhidrotic ectodermal dysplasias at the EDARADD locus. Hum Mutat 28(7):703-9.

Bayes M, Hartung AJ, Ezer S, Pispa J, Thesleff I, Srivastava AK, Kere J. 1998. The anhidrotic ectodermal dysplasia gene (EDA) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. Hum Mol Genet 7(11):1661-9.

Bohring A, Stamm T, Spaich C, Haase C, Spree K, Hehr U, Hoffmann M, Ledig S, Sel S, Wieacker P and others. 2009. WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. Am J Hum Genet 85(1):97-105.

Chassaing N, Bourthoumieu S, Cossee M, Calvas P, Vincent MC. 2006. Mutations in EDAR account for one-quarter of non-ED1-related hypohidrotic ectodermal dysplasia. Hum Mutat 27(3):255-9.

Dhanrajani PJ, Jiffry AO. 1998. Management of ectodermal dysplasia: a literature review. Dent Update 25(2):73-5.

Freire-Maia N. 1977. Ectodermal dysplasias revisited. Acta Genet Med Gemellol (Roma) 26(2):121-31.

Gat U, DasGupta R, Degenstein L, Fuchs E. 1998. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 95(5):605-14.

Gunadi, Miura K, Ohta M, Sugano A, Lee MJ, Sato Y, Matsunaga A, Hayashi K, Horikawa T, Miki K and others. 2009. Two novel mutations in the ED1 gene in Japanese families with X-linked hypohidrotic ectodermal dysplasia. Pediatr Res 65(4):453-7.

Headon DJ, Emmal SA, Ferguson BM, Tucker AS, Justice MJ, Sharpe PT, Zonana J, Overbeek PA. 2001. Gene defect in ectodermal dysplasia implicates a death domain adapter in development. Nature 414(6866):913-6.

Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B, Munoz F, Morgan D, Clarke A, Baybayan P and others. 1996. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. Nat Genet 13(4):409-16.

Koppinen P, Pispa J, Laurikkala J, Thesleff I, Mikkola ML. 2001. Signaling and subcellular localization of the TNF receptor Edar. Exp Cell Res 269(2):180-92.

Kumar A, Eby MT, Sinha S, Jasmin A, Chaudhary PM. 2001. The ectodermal dysplasia receptor activates the nuclear factor-kappaB, JNK, and cell death pathways and binds to ectodysplasin A. J Biol Chem 276(4):2668-77.

Lamartine J. 2003. Towards a new classification of ectodermal dysplasias. Clin Exp Dermatol 28(4):351-5.

Laurikkala J, Pispa J, Jung HS, Nieminen P, Mikkola M, Wang X, Saarialho-Kere U, Galceran J, Grosschedl R, Thesleff I. 2002. Regulation of hair follicle development by the TNF signal ectodysplasin and its receptor Edar. Development 129(10):2541-53.

Mikkola ML, Pispa J, Pekkanen M, Paulin L, Nieminen P, Kere J, Thesleff I. 1999. Ectodysplasin, a protein required for epithelial morphogenesis, is a novel TNF homologue and promotes cell-matrix adhesion. Mech Dev 88(2):133-46.

Monreal AW, Ferguson BM, Headon DJ, Street SL, Overbeek PA, Zonana J. 1999. Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. Nat Genet 22(4):366-9.

Monreal AW, Zonana J, Ferguson B. 1998. Identification of a new splice form of the EDA1 gene permits detection of nearly all X-linked hypohidrotic ectodermal dysplasia mutations. Am J Hum Genet 63(2):380-9.

Mou C, Jackson B, Schneider P, Overbeek PA, Headon DJ. 2006. Generation of the primary hair follicle pattern. Proc Natl Acad Sci U S A 103(24):9075-80.

Mustonen T, Ilmonen M, Pummila M, Kangas AT, Laurikkala J, Jaatinen R, Pispa J, Gaide O, Schneider P, Thesleff I and others. 2004. Ectodysplasin A1 promotes placodal cell fate during early morphogenesis of ectodermal appendages. Development 131(20):4907-19.

Nagy N, Wedgeworth E, Hamada T, White JM, Hashimoto T, McGrath JA. 2010. Schopf-Schulz-Passarge syndrome resulting from a homozygous nonsense mutation in WNT10A. J Dermatol Sci 58(3):220-2.

Nawaz S, Klar J, Wajid M, Aslam M, Tariq M, Schuster J, Baig SM, Dahl N. 2009. WNT10A missense mutation associated with a complete odonto-onycho-dermal dysplasia syndrome. Eur J Hum Genet 17(12):1600-5.

Paakkonen K, Cambiaghi S, Novelli G, Ouzts LV, Penttinen M, Kere J, Srivastava AK. 2001. The mutation spectrum of the EDA gene in X-linked anhidrotic ectodermal dysplasia. Hum Mutat 17(4):349.

Pinheiro M, Freire-Maia N. 1994. Ectodermal dysplasias: a clinical classification and a causal review. Am J Med Genet 53(2):153-62.

Priolo M, Silengo M, Lerone M, Ravazzolo R. 2000. Ectodermal dysplasias: not only 'skin' deep. Clin Genet 58(6):415-30.

Pummila M, Fliniaux I, Jaatinen R, James MJ, Laurikkala J, Schneider P, Thesleff I, Mikkola ML. 2007. Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of Bmp activity and induction of Shh expression. Development 134(1):117-25.

Schmidt-Ullrich R, Tobin DJ, Lenhard D, Schneider P, Paus R, Scheidereit C. 2006. NF-kappaB transmits Eda A1/EdaR signalling to activate Shh and cyclin D1 expression, and controls post-initiation hair placode down growth. Development 133(6):1045-57.

Shimomura Y, Wajid M, Weiser J, Kraemer L, Ishii Y, Lombillo V, Bale SJ, Christiano AM. 2009. Identification of mutations in the EDA and EDAR genes in Pakistani families with hypohidrotic ectodermal dysplasia. Clin Genet 75(6):582-4.

van der Hout AH, Oudesluijs GG, Venema A, Verheij JB, Mol BG, Rump P, Brunner HG, Vos YJ, van Essen AJ. 2008. Mutation screening of the Ectodysplasin-A receptor gene EDAR in hypohidrotic ectodermal dysplasia. Eur J Hum Genet 16(6):673-9.

van Geel M, Gattas M, Kesler Y, Tong P, Yan H, Tran K, Steijlen PM, Murrell DF, van Steensel MA. 2010. Phenotypic variability associated with WNT10A nonsense mutations. Br J Dermatol.

Vincent MC, Biancalana V, Ginisty D, Mandel JL, Calvas P. 2001. Mutational spectrum of the ED1 gene in X-linked hypohidrotic ectodermal dysplasia. Eur J Hum Genet 9(5):355-63.

Visinoni AF, Lisboa-Costa T, Pagnan NA, Chautard-Freire-Maia EA. 2009. Ectodermal dysplasias: clinical and molecular review. Am J Med Genet A 149A(9):1980-2002.

Yan M, Wang LC, Hymowitz SG, Schilbach S, Lee J, Goddard A, de Vos AM, Gao WQ, Dixit VM. 2000. Two-amino acid molecular switch in an epithelial morphogen that regulates binding to two distinct receptors. Science 290(5491):523-7.

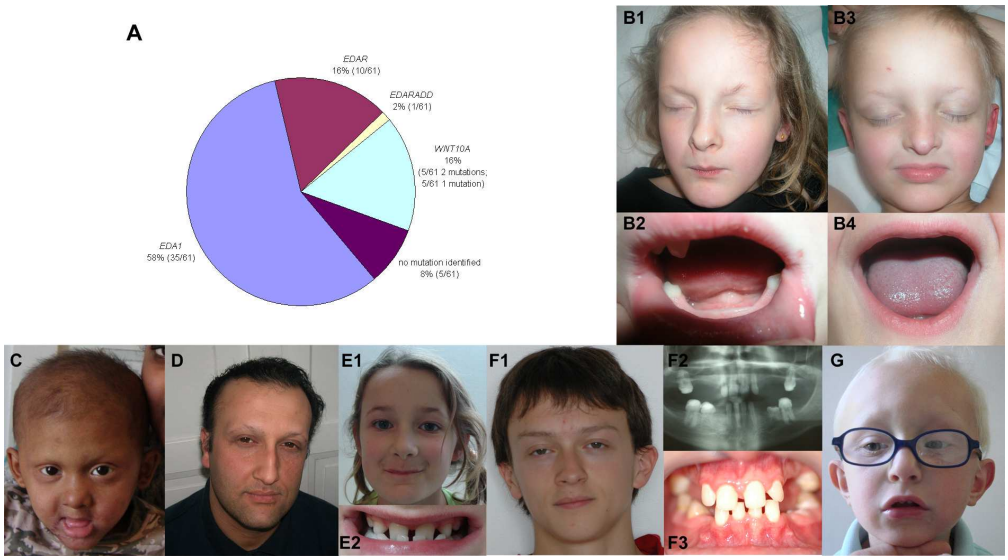

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Figure 2

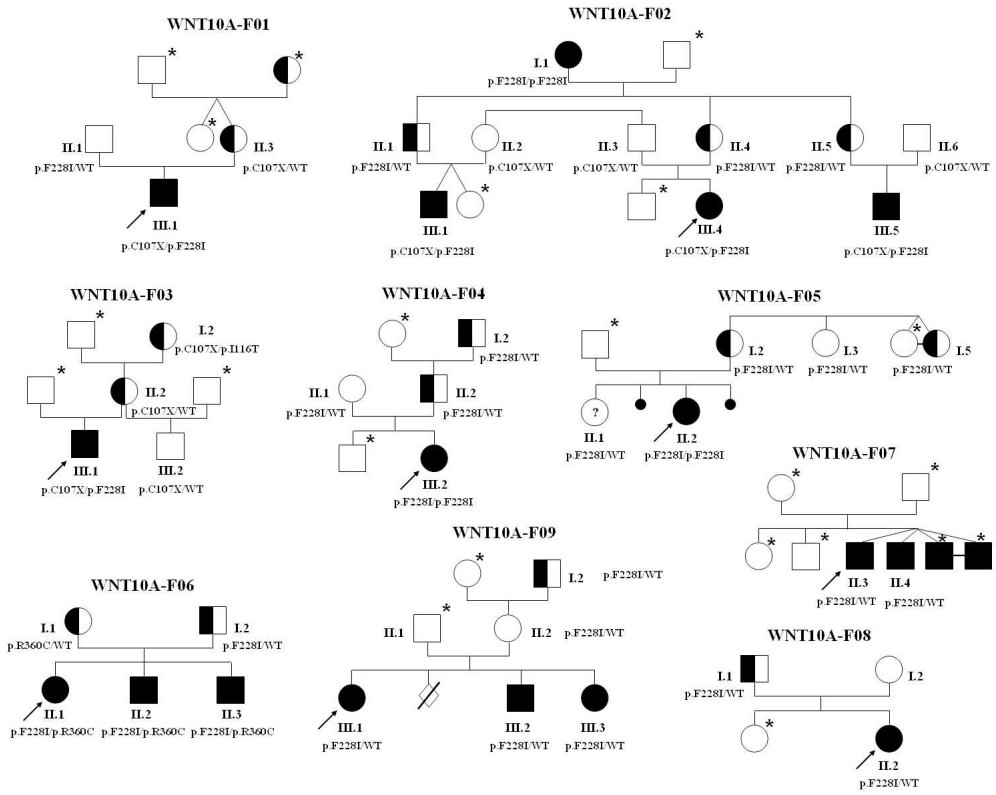

Figure 2
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Table 1: Clinical description and mutations in HED/EDA patients with mutations in *EDA1*, *EDAR* and *EDARADD* genes.

Proband's number	Sex, age (year)	Transmission, origin	Hair	Teeth	Sweating	Other signs	Nucleotide changes	Predicted proteic changes
***EDA1* (previously described mutations)**								
EDA1-F01	M, 10	Martinique	++	++	++	Eczema	c.466C>T (Bayes et al., 1998; Monreal et al., 1998)	p.R156C
EDA1-F02	M, 5	Algeria, CS	++	+++	++	Eczema, dry skin, facial dysmorphism	c.467G>A (Monreal et al., 1998)	p.R156H
EDA1-F03	M, 5	Tunisia	++	+++	+++	Eczema, dry skin	c.730C>T (Vincent et al., 2001)	p.R244X
EDA1-F04	F, 4	France	+	+ (C)	++	Dry skin, facial dysmorphism	c.764G>A (Pääkkönen et al., 2001)	p.G255D
EDA1-F05	M, 8	France	+++	++	+++	Facial dysmorphim	c.871G>C (Bayes et al., 1998)	p.G291R
EDA1-F06	M, 14	France	++	+++	+++	Eczema, facial dysmorphism	c.871G>A (Bayes et al., 1998)	p.G291R
EDA1-S01	M, 16	Spo, Yemen and Djibouti	+++	+++	++	Facial dysmorphism	c.871G>A (Bayes et al., 1998)	p.G291R
EDA1-F07	M, 28	France	++	++	+		c.871G>A (Bayes et al., 1998)	p.G291R
EDA1-F08	M, 13	France	+	+++	++	Dry skin, eczema, facial dysmorphism	c.892G>C (Bayes et al., 1998)	p.D298H
EDA1-S02	M, 4	Spo, Morroco, CS	+	+++	++	Nipples hypoplasia	c.895G>A (Monreal et al., 1998)	p.G299S
EDA1-F09	F, 28	France	++	++	+	Hypoplasia of the right nipple	c.895G>A (Monreal et al., 1998)	p.G299S
EDA1-F10	M, 3	France	+	+++	++	Eczema	c.1133C>T (Vincent et al., 2001)	p.T378M
EDA1-F11	M, 8	France	++	+++	++	Eczema	c.1141G>A (Gunadi et al. 2009, G>C)	p.G381R
EDA1-F12	M, 36	France	+++	+++	+++	Dry skin	c.789_825del (Bayes et al., 1998)	p.K263DfsX5
EDA1-F13	M, 10	France	+	++	+	Eczema	c.-181?_396+?del (Kere et al., 1996 ; Pääkkönen et al., 2001)	p.?

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Proband's number	Sex, age (year)	Transmission, origin	Hair	Teeth	Sweating	Other signs	Nucleotide changes	Predicted proteic changes
				***EDA1* (novel mutations)**				
EDA1-F14	F, 29	Algeria	+++	++	-	Eczema	c.2T>G	p.M1R
EDA1-F15	M, 31	France	++	+++	++		c.358G>T	p.E120X
EDA1-F16	F, 6	France	+	++	-	Dry skin, eczema	c.466C>G	p.R156G
EDA1-F17	M, 3	France	++	+++	+++		c.620G>T	p.G207V
EDA1-F18	M, 5	France	++	+ (C)	+	Dry mouth, facial dysmorphism	c.632C>G	p.T211R
EDA1-S03	M, 9	Spo, France/India	+++	+++	++	Eczema, facial dysmorphism	c.694C>T	p.Q232X
EDA1-F19	F, 10	France	++	++	+		c.797T>G	p.L266R
EDA1-F20	M, 14	France	+	+++	+	Dry skin, eczema	c.820T>A	p.W274R
EDA1-S04	M, 2	Spo, Congo	+++	+++	+++		c.878T>C	p.L293P
EDA1-F21	M, 6	France	+	++	-	Eczema	c.886C>G	p.L296V
EDA1-S05	M, 11	Spo, France	++	+++	++		c.896G>A	p.G299D
EDA1-S06	M, 33	Spo, France	++	+++	+	Palmar keratoderma, facial dysmorphism	c.968T>G	p.V323G
EDA1-S07	M, 16	Spo, France	+++	+++	++	Dry skin, eczema	c.1037G>A	p.C346Y
EDA1-S08	M, 6	Spo, France	+	+	+		c.1067C>T	p.A356V
EDA1-F22	M, 34	France	+++	+++	+++		c.397-1G>A	p.?
EDA1-F23	M, 6	Japan	++	+++	++	Dry skin, facial dysmorphism	c.925-1G>C	p.?
EDA1-F24	F, 43	France	++	++	++		c.361delG	p.A121PfsX16
EDA1-F25	M, 3	Japan	+	+++	++	Eczema, no lacrimation	c.640dupA	p.M214NfsX26
EDA1-F26	M, 5	France	+	+++	+	Eczema	c.573_590del	p.G192_Q197del
EDA1-F27	M, 11	Portugal	++	+++	+	Dry skin, facial dysmorphism	c.503-?_1176+?del	p.?

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Proband's number	Sex, age (year)	Transmission, origin	Hair	Teeth	Sweating	Other signs	Nucleotide changes	Predicted proteic changes
				EDAR				
EDAR-S01	M, 7	Spo, AR, Portugal	++	+++	++	Eczema	c.[266G>A]+[442+1G>A] (Monreal et al. 1999; Chassaing et al. 2006)	p.R89H + ?
EDAR-F01	M, 3	AR, Pakistan, CS	+	+++	++		c.[1073G>A]+[1073G>A] (Shimomura et al., 2009)	p.R358Q + p.R358Q
EDAR-F02	M, 11	AR, Kuwait, CS	++	++	++		c.[1208C>T]+[1208C>T] (Chassaing et al. 2006)	p.T403M + p.T403M
EDAR-F03	F, 8	AD, France	++	++	+	Facial dysmorphism	c.1222A>T	p.I408F
EDAR-F04	M, 17	AD, Sefarad	+	+	+	Facial dysmorphism	c.1259G>A (Monreal et al. 1999)	p.R420Q
EDAR-F05	M, 34	AD, Algeria, CS	++	+	+	Dry skin	c.1259G>A (Monreal et al. 1999)	p.R420Q
EDAR-S02	M, 3	Spo, AR, Turkey, CS	+++	+++	+++	Facial dysmorphism, diffuse palmoplantar hyperkeratosis	c.[156_157delinsC]+[156_157delinsC]	p.G53EfsX50
EDAR-S03	F, 37	Spo, AD, Portugal	+	++	+		c.875_876del	p.P292RfsX52
EDAR-F06	F, 10	AD, Sefarad	++	+++	+	Eczema, dry skin	c.1049_1052del	p.D351AfsX20
EDAR-F07	M, 6	AD, Portugal, CS	+	+	+		c.1186_1188dup	p.Q396dup
				EDARADD				
EDARADD-F01	F, 11	AD, France	+	+	+	Dry skin, abnormal nails, palmo-plantar keratoderma	c.328G>T	p.D110Y

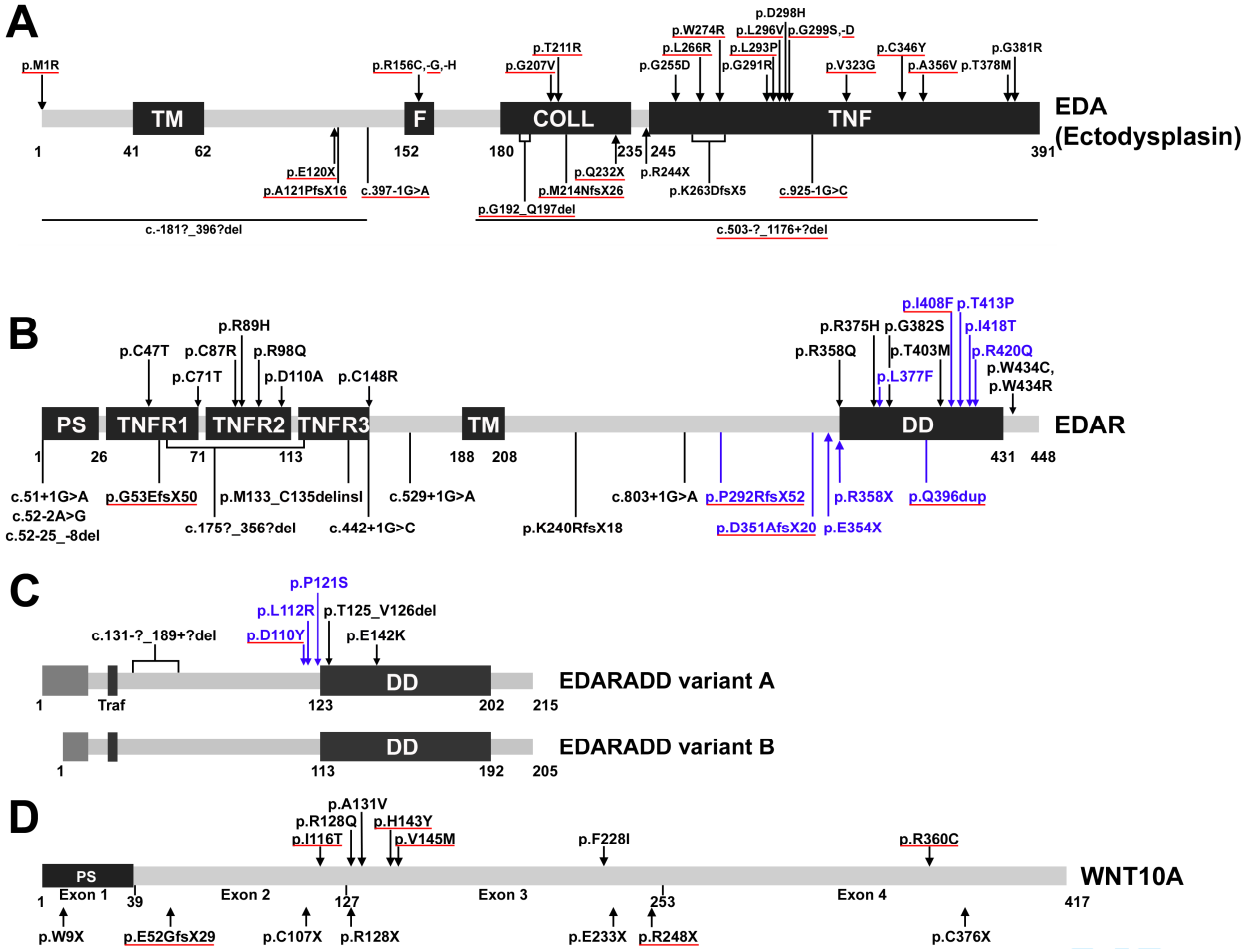
Mutations are annotated with +1 corresponding to the A of the ATG translation initiation codon, or with the initiation codon as codon 1 in the GenBank reference sequences (NM_001399.4, and NP_001390.1 for *EDA1*; NM_022336.3, and NP_071731.1 for *EDAR*; NM_080738.3 and NP_080738.3 for *EDARADD*). For the three genes: M male; F female; Spo sporadic (all other cases are familial), CS consanguinity, AR autosomal recessive, AD autosomal dominant; +, ++, and +++ degree of severity of present feature, - within normal clinical limits; C conical teeth.

Table 2: Clinical description of patients with mutations in *WNT10A* gene.

Proband's number	Patient	Sex, Age (year)	Origin	Hair	Teeth	Sweating	Other signs	Nucleic changes	Predicted proteic changes
HED/EDA phenotype									
WNT10A-F01	II.1	M, 46	France	-	-	-		c.682T>A	p.F228I
	II.3	F, 44	France	-	A	-		c.321C>A	p.C107X
	III.1	**M, 17**	**France**	**-**	**A+**	**Hypo**		**c.[321C>A]+[682T>A]**	**p.C107X + p.F228I**
WNT10A-F02	I.1	F, 70	France	-	A	Hypo		c.[682T>A](+)[682T>A]	p.F228I + p.F228I
	II.1	M, 49	France	-	D	-		c.682T>A	p.F228I
	II.2	F, 45	France	-	-	-		c.321C>A	p.C107X
	II.3	M, 46	France	-	-	-		c.321C>A	p.C107X
	II.4	F, 44	France	-	Ed	Hyper		c.682T>A	p.F228I
	II.5	F, 46	France	-	Ed, Sh	Hyper	Dry skin	c.682T>A	p.F228I
	II.6	M, 50	France	-	-	-		c.321C>A	p.C107X
	III.1	M, 18	France	-	A	-	Palmo-plantar keratoderma	c.[321C>A]+[682T>A]	p.C107X + p.F228I
	III.4	**F, 16**	**France**	**-**	**A**	**-**		**c.[321C>A]+[682T>A]**	**p.C107X + p.F228I**
	III.5	M, 24	France	-	A, D	-		c.[321C>A]+[682T>A]	p.C107X + p.F228I
WNT10A-F03	I.2	F, 63	France	-	D	Hypo		c.[321C>A]+[347T>C]	p.C107X + p.I116T
	II.2	F, 41	France	-	D	Hypo		c.321C>A	p.C107X
	III.1	**M, 18**	**France**	**T**	**A+**	**Anh**	**Dry eyes, otitis**	**c.[321C>A]+[682T>A]**	**p.C107X + p.F228I**
	III.2	M, 14	France	-	-	-		c.321C>A	p.C107X
WNT10A-F07	**II.3**	**M, 44**	**France**	**Eb**	**A**	**Hypo**		**c.682T>A**	**p.F228I**
	II.4	M, 44	France	Eb	A	Hypo		c.682T>A	p.F228I
WNT10A-F08	I.1	M, 42	France	-	C, Ed	-		c.682T>A	p.F228I
	II.2	**F, 13**	**France**	**T**	**A**	**Ih**		**c.682T>A**	**p.F228I**
WNT10A-F09	I.2	M, 77	France	-	-	Ih		c.682T>A	p.F228I
	II.2	F, 42	France	-	-	-		c.682T>A	p.F228I
	III.1	F, 16	France	-	M, A+	-		c.682T>A	p.F228I
	III.2	**M, 12**	**France**	**-**	**M, A+**	**Ih**	**Inverted nipples, periorbital pigmentation**	**c.682T>A**	**p.F228I**
	III.3	F, 9	France	-	M, A+	-		c.682T>A	p.F228I
WNT10A-S01		**F, 29**	**Turkey, CS**	**T**	**M, A**	**Anh**	**Follicular keratosis**	**c.[433G>A](+)[433G>A]**	**p.V145M + p.V145M**
WNT10A-S02		**F, 18**	**China, CS**	**T**	**M, A**	**Hypo**	**Palmoplantar keratoderma**	**c.[742C>T](+)[742C>T]**	**p.R248X + p.R248X**
WNT10A-S03		**F, 9**	**France**	**Eb**	**M, A**	**Hyperthermia upon infections**	**Dry skin**	**c.427C>T**	**p.H143Y**
WNT10A-S04		**M, 6**	**France**	**T**	**M, A, Sh**	**Hypo**		**c.682T>A**	**p.F228I**

Proband's number	Patient	Sex, Age (year)	Origin	Hair	Teeth	Sweating	Other signs	Nucleic changes	Predicted proteic changes
OODD syndrome									
WNT10A-S05		**M, 35**	**France**	**Eb**	**A**	**-**	**Slight onychodysplasia, palmoplantar keratoderma**	**c.146dupT**	**p.E52GfsX29**
OODD-like syndrome									
WNT10A-F04	I.2	M, 89	France	Sp	?	?	Thick nails	c.682T>A	p.F228I
	II.1	F, 57	France	?	?	?		c.682T>A	p.F228I
	II.2	M, 48	France	-	S	-		c.682T>A	p.F228I
	III.2	**F, 24**	**France**	**-**	**M,A+, C, D**	**-**	**Split nails, eczema**	**c.[682T>A]+[682T>A]**	**p.F228I + p.F228I**
WNT10A-F05	I.2	F, 66	France	-	-	-		c.682T>A	p.F228I
	I.6	F, 58	France	-	Ed	-		c.682T>A	p.F228I
	II.1	F, 29	France	-	PT	-		c.682T>A	p.F228I
	II.2	**F, 25**	**France**	**T**	**M,A+**	**-**	**Nail dystrophy**	**c.[682T>A](+)[682T>A]**	**p.F228I + p.F228I**
Unclassified ED									
WNT10A-F06	I.1	F, 50	France	T	-	-	Eczema	c.1078C>T	p.R360C
	I.2	M, 47	France	-	Ed	Hyperhidrosis	Delayed puberty	c.682T>A	p.F228I
	II.1	F, 20	France	T	A	-		c.[682T>A]+[1078C>T]	p.F228I + p.R360C
	II.2	**M, 17**	**France**	**T**	**A+, Ed**	**Hyperhidrosis upon infections**	**Soft nails, eczema, short stature (-2DS), delayed puberty**	**c.[682T>A]+[1078C>T]**	**p.F228I + p.R360C**
	II.3	M, 13	France	-	A-	Hyperhidrosis upon infections	Eczema	c.[682T>A]+[1078C>T]	p.F228I + p.R360C

Mutations are annotated with +1 corresponding to the A of the ATG translation initiation codon, or with the initiation codon as codon 1 in the GenBank reference sequences (NM_025216.2, and NP_079492.2 for *WNT10A*). Probands (in bold) and their relatives figure in the table, all numbered from their pedigree presented in Figure 2. M male; F female; WNT10A-F01 to F09 are familial cases, WNT10A-S01 to S05 are sporadic; CS consanguinity; - within normal clinical limits, ? unknown information; A agenesis, A+ severe agenesis, M microdontia, Ed enamel dysplasia, D persistant decidual teeth, C conical teeth, Sh abnormal tooth shape, PT palatal tooth; T thin and fragile hair, Eb rare eyebrows, Sp sparse hair; Ih intolerance to heat, Hypo hypohidrosis and Anh anhidrosis.



Supp. Figure S1: Distribution of previously known and newly identified mutations regarding to functional domains of EDA1 (A), EDAR (B), EDARADD (C) and WNT10A proteins (D). Missense mutations are indicated above the schematic representation of each protein, whereas nonsense, splice and truncating mutations are positioned underneath. Mutations with a dominant mode of inheritance in EDAR and EDARADD figure in blue; newly identified mutations are underlined in red. Ectodysplasin is mainly mutated in its furin cleavage site, its collageneous and its TNF domains. Mutations in EDAR are located either in the N-terminal ligand binding domain, or in the C-terminal Death Domain (DD). Interestingly, all dominant EDAR mutations are located in its C-terminal region. All EDARADD missense mutations are located in or very close to its Death Domain. TM: transmembraneous domain; F: furin cleavage site; COLL: collagen domain; TNF: TNF-like domain; PS: peptide signal; TNFR1 to -3: TNF Receptor like domain; DD: Death domain. Reference sequences used to annotate all mutations are: NP_001390.1 (EDA1), NP_071731.1 (EDAR), NP_080738.3 (EDARADD), NP_079492.2 (WNT10A).

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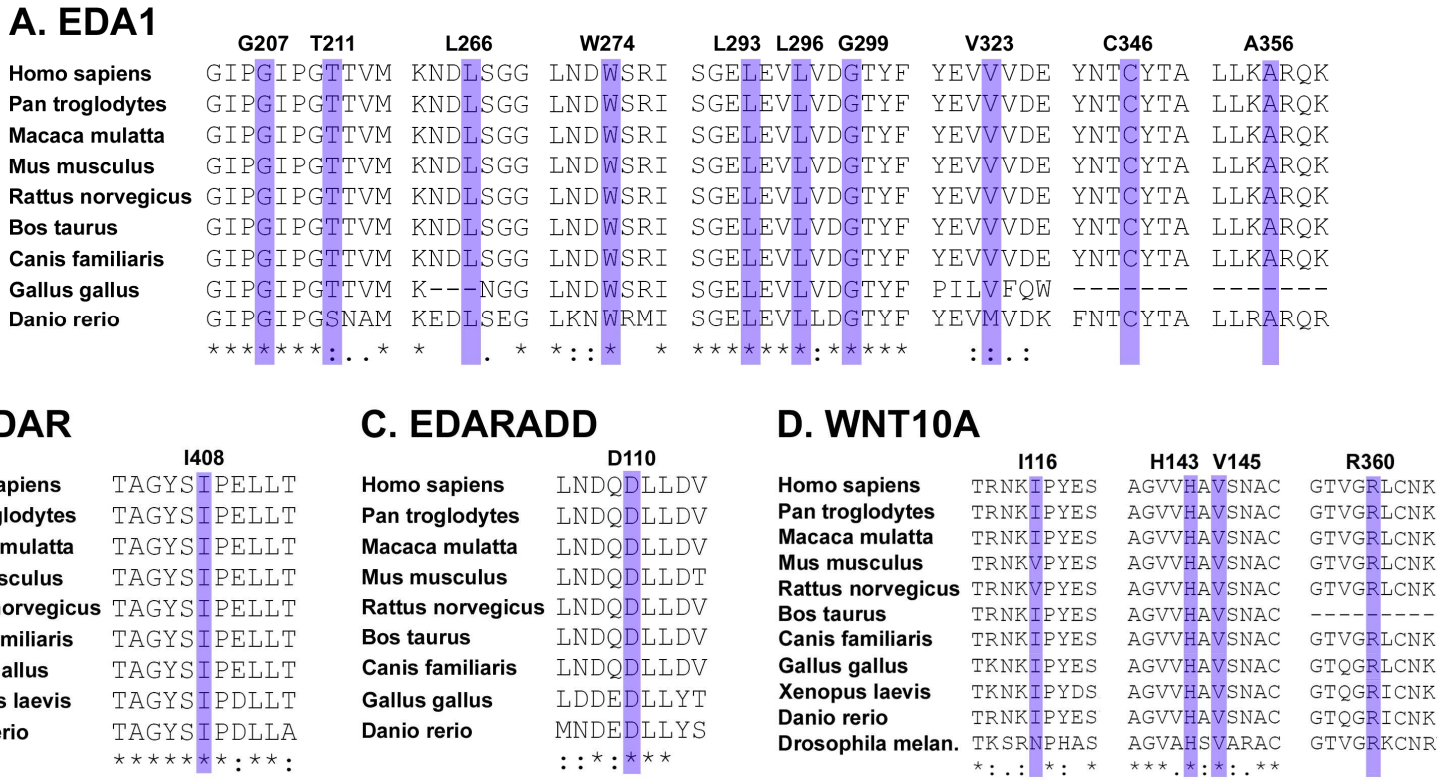
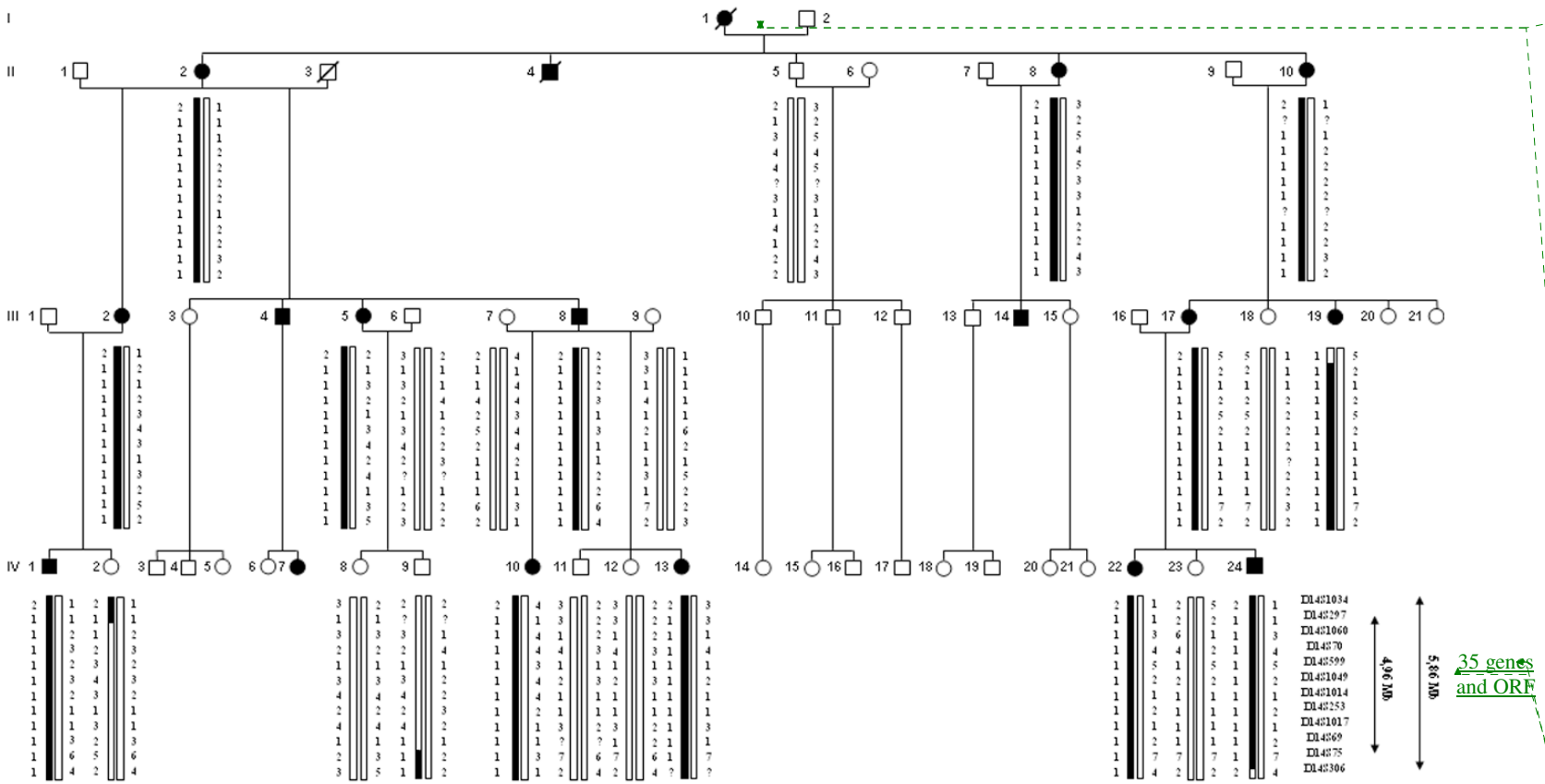


Supp. Figure S2: Evolutionary conservation of residues with novel missense mutations in EDA1, EDAR, EDARADD and WNT10A proteins.
Alignments were perfoemd using Clustalw program, using a BLOSUM matrix.
(A) Conservation of the 10 novel missense mutations in EDA1: sequences used for the alignment are NP_001390.1 (Homo sapiens), XP_529025.2 (Pan troglodytes), XP_001082424.1 (Macaca mulatta), NP_034229.1 (Mus musculus), XP_228582.5 (Rattus norvegicus), NP_001075212.1 (Bos taurus), NP_001014770.1 (Canis lupus familiaris), XP_420158.1 (Gallus gallus), NP_001108537.1 (Danio rerio).
(B) Conservation of the novel missense mutation on residue I408 in EDAR: sequences used for the alignment are NP_071731.1 (Homo sapiens), XP_001139583.1 (Pan troglodytes), XP_001084259.1 (Macaca mulatta), NP_034230.1 (Mus musculus), XP_345110.4 (Rattus norvegicus), XP_538426.2 (Canis familiaris), NP_001012629.1 (Gallus gallus), NP_001080516.1 (Xenopus laevis), NP_001108536.1 (Danio rerio).
(C) Conservation of the novel missense mutation on residue D110 in EDARADD: sequences used for the alignment are NP_542776.1 (Homo sapiens), XP_514291.2 (Pan troglodytes), XP_001099801.1 (Macaca mulatta), NP_598398.3 (Mus musculus), XP_574055.3 (Rattus norvegicus), XP_596273.2 (Bos taurus), XP_849101.1 (Canis familiaris), NP_001012405.2 (Gallus gallus), XP_695138.3 (Danio rerio).
(D) Conservation of the four novel missense mutations in WNT10A: sequences used for the alignment are NP_079492.2 (Homo sapiens), XP_516098.2 (Pan troglodytes), XP_001095740.1 (Macaca mulatta), NP_033544.1 (Mus musculus), NP_001101697.1 (Rattus norvegicus), NP_001092548.1 (Bos taurus), XP_545648.2 (Canis familiaris), NP_001006590.1 (Gallus gallus), ABG49498.1 (Xenopus laevis), NP_571055.1 (Danio rerio), NP_609109.2 (Drosophila melanogaster).



Supp. Figure S3: Haplotype analysis of the HED family for polymorphic markers on chromosome 14q12-13.1. Four recombinant events occurred in individuals III.19, IV.2, IV.9 and IV.24. Filled symbols represent affected individuals. The common disease-associated haplotype is shown in black.

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Supp. Table S1: Sequences of the primers used for direct sequencing of *EDA1*, *EDAR*, *EDARADD* and *WNT10A* genes, and QMPSF analysis for *WNT10A* gene.

Genes	Exons	Forward primers (5'-3')	Reverse primers (5'-3')
EDA1	1	CGGAGTAGAGCTGCACATGCG	CCAGGGCAGGTTGTCTTCGGT
	3	GGAGGGGAAGATGGGCTCAG	TGGTGGCTCACGCCTGTAAT
	4	AGGAGTCAGAAGACAGAATGG	AAGGGCAGGGAGAAGAACAAG
	5	AGATCGTGCCACTGAACTCC	GCTCTCAGGATCACCCACTC
	6	CCACTGAAGATGAAGGTCAGG	GCAAGACACCCTTTCCTTAGC
	7	GGTCACATAGCTAGGAAGCGG	CTTTCAGCTCCGTCATCAGTG
	8	CAGGCCTGGCAGCTGCTTTAC	TGGCCCCCTCTCTCTTTCCTC
	9	GAACAATGCCTGTCACCTGTC	AAGTCAAGCAGGCCTTGTCAC
EDAR	2	CAGAGTCAGCCCAAGTGGCAT	GCTGTGTGTATCACACCACAAAC
	3	CCAGGTGATCAACCAGGAGCC	ATGAATGCTTAGCTGGTGAGTGC
	4	AGACAGCTGGCACGTCACT	ACAGGGGTCATGGATACTGC
	5	CTGAGTGGACAGAGCAGGTG	AAGGCTCAGATGTGGCAAAC
	6	CAATAACGATGACTCTTTAGGG	GAGTTGATCCCTCTATGGGTG
	7-8	CCAGCGCGGAGGATTTGGTTC	CAGTATGGTTCAGCATGTGAGAG
	9	TGCTTGTGCTCTCACATGCT	CCAGTCAGCAAAGAGGTGGT
	10	GTGCCCACAAGGTGCCCAGT	CCCGTCTTGCAGGAGAGCTGA
	11	AGTCTGACCACCCAGCTGAGC	ATGCCTTCCGATATCTGGGAAC
	12	CCTTCTATTGACTGTGACTTGCAACA	AGCTCCAGAGCCCTCGTTGG
EDARADD	1a	GAAAGAACCACAAACCAAACC	TGCCTTCACACATAAGAACAG
	1b	AGGTACCGAGGGACGCGC	GTTTGCAGGACGTGTCTCAAC
	2	AGTAAGGTTTTCTTCAGCCTAAG	CCAGGGAAGTGGGTAAAGCC
	3	CCTTGATTTCATTCCTGTCGA	GTCACGAGCTAATCTATGGGC
	4	ATCCTTAAGAGCAGAGTTTGG	CTGTTTATGATCTAGAAATCCTG
	5	GCGCTCAAGGTGCTCGTATTCT	TTACAGGCGCCCACCACAACC
	6-1	TATGATGCTTTTGACAATTCAGCA	ACGAGCACAATTCGTCATAGGACA
	6-2	CGTGTCACCCAACGGTGAAAA	CCCCTCCACAAAACTGCCAGC
WNT10A	1	GAGTCGGAGCTGTGTGTCG	GAGCTCACTGCCTTTGGTTC
	2	CTGGGCAGGATGATTGTGAG	CTGAGATCAGAAAGAGGAAGG
	3	TGATTTCTGCCCTTCTTTGAC	TGCACAGTGCATACTCAGTG
	4	GGTACAGAAGTTCTTCTGACTG	AGAAGTGAGTGGTGGGGTTC
WNT10A-QMPSF	1	GAGTCGGAGCTGTGTGTCG	FAM-AGCGCTGGCCGCGGCTGG
	2	FAM-CTATGAGAGTCCCATCTTCAGC	CTGAGATCAGAAAGAGGAAGG
	3	TGATTTCTGCCCTTCTTTGAC	FAM-CGATGGCGTAGGCAAAAGCG
	4	GGTACAGAAGTTCTTCTGACTG	FAM-CCACGAAACAGCACCAGTGG
GFAP-QMPSF	3	GAGGAAAGGATTGATGGCCA	FAM-AAGAACCGGATCTCCTCCTC
MLH1-QMPSF	18	FAM-GTAGTCTGTGATCTCCGTTT	ATGTATGAGGTCCTGTCCTA

Supp. Table S2: Clinical description of non-mutated patients.

Proband's number	Gender, age (year)	Ethnical origin	Hair	Teeth	Sweating	Other signs
HED-F01	F, 18	France	++	+++	+++	Onychodystrophy
HED-F02	M, 11	Portugal	++	+	+++	Facial dysmorphism
HED-F03	M, 19	France	+++	++	+++	Telangiectasia on the face
HED-S01	M, 10	Spo, France	-	+++	++	
HED-F04	M, 10	France	++	++	++	

M male; F female; Spo sporadic (all other cases are familial); +, ++, and +++ degree of severity of present feature, - within normal clinical limits.

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